

Reproductive behavior of *Chavesincola inexpectabilis* (Opiliones, Gonyleptidae) with description of a new and independently evolved case of paternal care in harvestmen

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Abstract. In this paper, we investigate the reproductive behavior of the gonyleptid *Chavesincola inexpectabilis* Soares & Soares 1946 (Heteropachylinae) and provide basic descriptive information about courtship, copulation, oviposition, and paternal care. Like most gonyleptids, males of *C. inexpectabilis* have a strong armature on the fourth pair of legs and use their spines and apophyses to fight other males and to repel them from their nesting sites. The mating pair interacts briefly before copulation, but the male touches the female both during and after penetration while she oviposits. The oviposition behavior differs markedly from that of other Laniatores: females hold the eggs on the chelicerae before depositing them on the substrate. After oviposition, the eggs are left under the guard of the male to defend against attack from cannibalistic conspecifics. Mapping the available data on reproductive biology of the Gonyleptidae on the phylogeny of the family, it is possible to infer that paternal care has evolved at least three times independently: once in the clade Progonyleptoidellinae + Caelopyginae, once in the Gonyleptinae, and once in the Heteropachylinae, which occupies a basal position within the group.

Keywords: Copulation, courtship, evolution, Heteropachylinae, oviposition, sexual dimorphism

The great majority of the harvestmen species reproduce sexually, although some species reproduce asexually by parthenogenesis (e.g., Phillipson 1959; Tsurusaki 1986). Fertilization is internal and the transfer of sperm may occur indirectly through spermatophores in representatives of the suborder Cyphophthalmi, or directly by means of a long and fully intromittent male genitalia in the suborders Eupnoi, Dyspnoi, and Laniatores (Machado & Macías-Ordóñez 2007). Courtship before intromission is generally quick and tactile, but there are some cases in which males offer a glandular secretion produced in their chelicerae before copulation as a nuptial gift for their mates. Courtship during intromission, on the other hand, may be longer and involve leg tapping and rubbing. Copulation is often followed by a period of mate guarding in which the female is held or constantly touched by the male (see table 12.1 in Machado & Macías-Ordóñez 2007).

Females may lay their eggs immediately or in the months after copulation, and the oviposition strategies seem to be related to the length of the ovipositor. Most species of the suborders Cyphophthalmi and Eupnoi have a long ovipositor and hide their eggs inside small holes in the soil, trunk crevices, or under stones. Representatives of the suborders Dyspnoi and Laniatores, constrained by their short ovipositor, lay their eggs on exposed substrates such as leaves, wood, and rocks (Machado & Macías-Ordóñez 2007). The forms of parental care range from microhabitat selection for oviposition to active egg guarding by a parental individual. In most species, eggs are laid singly in shallow natural cavities or are covered by debris by the female. In some species, however, females lay eggs in a single large clutch and brood eggs throughout the embryonic development, remaining with the newly hatched nymphs for some days until they disperse (Machado & Raimundo 2001). Maternal care has been reported for many families of the suborder Laniatores, especially among the

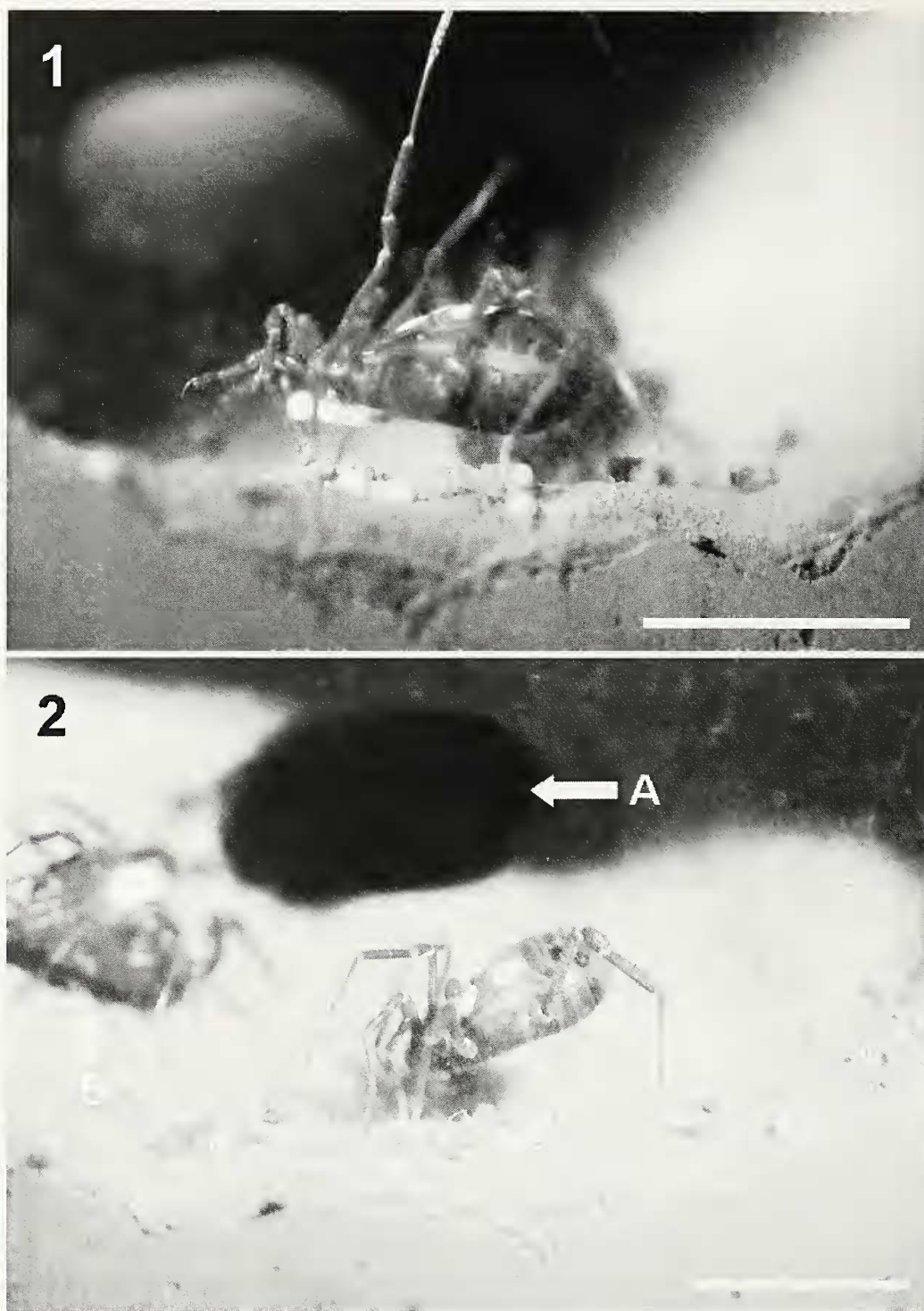
Neotropical representatives of the superfamily Gonyleptoidea (see Machado & Warfel 2006).

While maternal egg guarding is widespread among arachnids, exclusive paternal care is present only in the order Opiliones (Machado et al. 2004). Male assistance has evolved in at least five families belonging to three non-closely related superfamilies of the suborder Laniatores: Travunioidea, Epedanoidea, and Gonyleptoidea (Machado 2007). Within Gonyleptidae, which comprises nearly 1,000 species and corresponds to the largest family of Laniatores, there are eight cases of paternal care recorded so far (Machado & Macías-Ordóñez 2007). In this paper, we investigate the reproductive behavior of the gonyleptid *Chavesincola inexpectabilis* Soares & Soares 1946 (Heteropachylinae) and provide basic descriptive information about courtship, copulation, oviposition, and paternal care of this species. This study is the first description of the reproductive biology of a representative of the subfamily Heteropachylinae and the results obtained here represent a new and independently evolved case of paternal care in gonyleptid harvestmen.

METHODS

In all, 9 females and 14 males of *C. inexpectabilis* were collected along the borders of a small (ca 8 ha) urban forest fragment in Santa Teresa city (19°58'S; 40°32'W; elev. 675 m), Espírito Santo state, southeastern Brazil. The individuals were found under rotting logs and piles of tree fern trunks discarded from a green house nearby. They were brought to our laboratory in the Natural History Museum at Universidade Estadual de Campinas (São Paulo state, Brazil) and were maintained in a communal terrarium (40 × 90 cm base, 20 cm height) containing soil, small pieces of tree fern trunks collected in the study site, and 10 artificial nests built in clay blocks (with 6 × 2 cm base, 3 cm height). Each mud nest had a central hole (1 cm in diameter and 2 cm depth) crossing the clay block from side to side. These blocks were placed against

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Figures 1, 2.—1. Marked female of the harvestman *Chavesincola inexpectabilis* everting the ovipositor and manipulating the egg with the chelicerae while scrapping the substrate of the nest with her first pair of legs. 2. Another marked female covering a recently laid egg with debris. Behind the female, it is possible to see the nest entrance (A) and the guarding male walking around while she is ovipositing (B). Both photos were taken through the glass wall of the terrarium. Scale bars = 5 mm.

the glass wall of the terrarium so that it was possible to observe the harvestman behavior inside the nests through the glass (Figs. 1, 2). These mud nests simulated natural cavities in roadside banks, which are occupied by males of another Heteropachylinae species from Espírito Santo (*Pseudopucroliia* sp.). Males of *Pseudopucroliia* take care of the eggs laid by females inside these natural cavities, and the possession of

nests is crucial for their reproductive success (Nazareth & Machado unpubl. data). During the study period, the abiotic conditions in the laboratory were (mean \pm SD): temperature of $25.5 \pm 1.2^\circ$ C, humidity of $82.0 \pm 5.4\%$, and photoperiod of 13L:11D.

Individuals were measured (dorsal scute width) and individually marked on their dorsal scute with colored dots

of enamel paint. They were fed pieces of dead cockroaches and an artificial diet for ants (Bhatkar & Whitcomb 1970) three times a week. The mud nests were individually numbered and, at each observation, the identity of the individuals inside each nest was recorded. Behavioral data are based on nearly 50 h of ad libitum observations (sensu Altman 1974), of which 43 h were conducted at night (from 18:00 to 00:00 h) when individuals were more active. Nocturnal observations were made with a red lamp to avoid disturbing the animals (cf. Elpino-Campos et al. 2001; Pereira et al. 2004). Continuous recording (sensu Martin & Bateson 1993) was made of all relevant behavioral events such as fights between males, copulations, and ovipositions. Voucher specimens of males and females were deposited in the arachnological collection of the Museu de Zoologia da Universidade de São Paulo (MZSP), São Paulo state, Brazil.

RESULTS

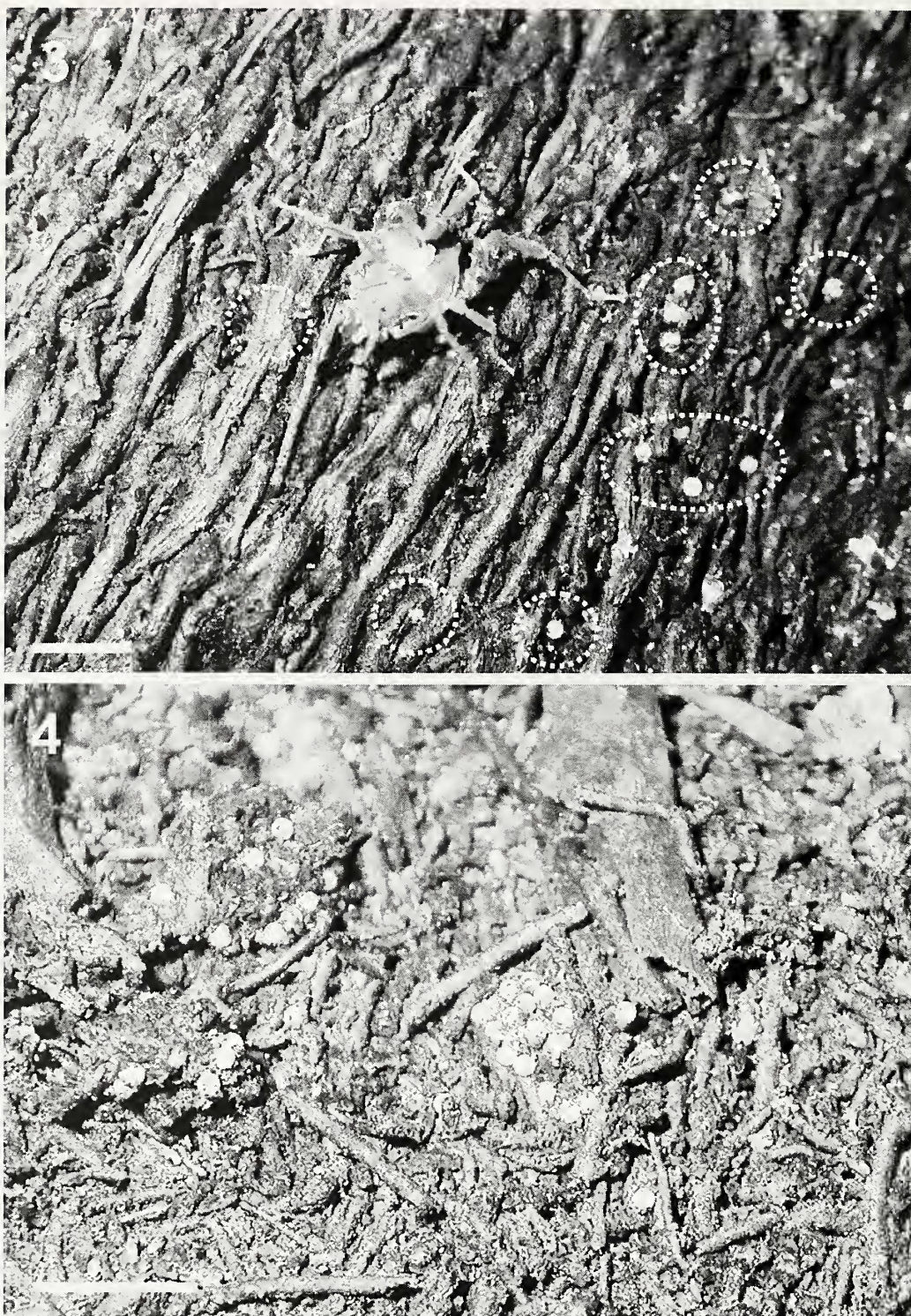
Nesting.—Ten males were observed occupying and occasionally fighting for the ownership of the mud nests. No fight or any kind of aggressive interaction was observed between males outside the nests. Only two males were observed mating: the first one (M1) achieved copulation after staying in the same mud nest for four consecutive days, and the second (M2), after five consecutive days. These males had a dorsal scute width of 4.99 mm (M1) and 4.71 mm (M2), and were, respectively, the first and the third largest males in the terrarium (mean male size \pm SD = 4.48 ± 0.27 mm; $n = 14$). On two occasions, as soon as an intruder male entered a mud nest occupied by one of these two males, a brief period of intense mutual tapping with the second pair of legs occurred. After that, the individuals turned their backs to each other and intertwined the fourth pair of legs, which bears many spines and tubercles. In this position, the males seemingly attempted to capsize each other by means of sudden upward movements in which each male brought its femur IV close to the body, pinching his opponent's fourth pair of legs, a behavior known as "nipping 2" (sensu Willemart et al. 2009). This phase of nipping 2 lasted nearly 30 s in the two fights observed and, in both cases, resident males managed to pull the intruders out of the mud nests.

Copulation.—All copulations occurred inside nests, and no sexual interaction between males and females were observed in other places of the terrarium. Most of the females (6 out of 9) were observed copulating at least once. One of them was observed copulating and laying eggs with M1 and M2 and another one was observed laying eggs twice with M1. M1 copulated at least five times with four different females, resulting in a total of 228 eggs in his mud nest, and M2 copulated at least three times with three different females, resulting in a total of 83 eggs. After the hatching of all nymphs inside his nest, M1 left the mud nest and established a new nesting site under a piece of tree fern trunk (Fig. 3). After 11 days, 54 eggs covered by debris (Fig. 4) and in two different stages of embryonic development (according to Machado et al. 2004) were found attached to the undersurface of the tree fern trunk. Since there was no egg under the tree fern trunk before M1 arrival, the presence of the clutch suggests that M1 copulated with two females or twice with the same female. M1 remained close to the eggs in the trunk nest until they hatched 16 days later.

Just before copulation, the male approached the female frontally and intensely tapped her genital opening with his second pair of legs. Meanwhile, the male also gently touched the dorsum of the female with his first pair of legs ($n = 2$). In one case, touching behavior lasted 30 s and, in the sequence, the male (M1) grasped the female pedipalps with his own pedipalps. The female raised the front of her body, exposing her ventral region to the genital opening of the male. In this position, the male everted his penis and penetrated the female's genital opening. The other courtship lasted almost 1 h and, during all this time, the male (M2) touched the female as described above. During most of the courtship, the female bent the front of her body so that it was impossible for the male to penetrate her. Occasionally, she also put her venter in contact with the substrate, also preventing the male from touching her genital opening. Eventually, the male managed to grasp the female pedipalps with his pedipalps and then she spontaneously raised the front of her body allowing penetration.

Both copulations lasted nearly 2 min, and during penetration, the male performed intense leg tapping on the dorsum of the female using his first pair of legs, and simultaneously on the female's hind legs and venter using his second pair of legs. Penetration was apparently terminated by the female when she was able to propel herself backwards with enough force to release herself from the grasp of the male's pedipalps. Immediately after separation, males continued to tap the dorsum and venter of their partners with the second pair of legs for nearly 2 min.

Oviposition.—After copulation, the female generally walked inside the mud nest for nearly 3 min ($n = 7$), always followed by the male, probably searching for a proper place for egg laying. In the first step of the oviposition, the female everted her ovipositor and placed its tip in contact with her chelicerae for up to 7 min. At the same time, the male, stood behind the female, repeatedly tapped her dorsum using his second pair of legs. Once every 3 min, the male also gently tapped the venter of the female ($n = 2$ ovipositions); it was not possible to see if the male touched the ovipositor. Next, the female released an egg, which was held on the chelicerae while she scraped the nest's wall with her first pair of legs (Fig. 1). Every two or three scrapes of the nest's wall, the female brought the leg to the mouth, probably to clean or humidify the tip of the leg; this process lasted from 7 to 13 min. In the sequence, the female put the egg on the scraped area using her chelicerae and rolled it on the substrate using the first pair of legs until the egg was completely covered by debris, a process that lasted up to 1 min (Fig. 2). After oviposition of each egg, the male walked around inside the nest until the female started to lay the next egg (Fig. 2). At this moment, the male resumed tapping the female using his second pair of legs, as described above. The whole process of oviposition lasted 2 to 4 days (mean \pm SD = 2.6 ± 0.7 ; $n = 8$), and was interrupted by periods of rest (sensu Elpino-Campos et al. 2001), when both male and female did not interact with each other. After this period, the female abandoned the nest and the eggs were left under the male protection until they hatched 23–24 days later. The mean number of eggs laid in each oviposition was 38.9 (SD = 12.2; $n = 8$), and the intervals between the two oviposition events of each female ranged from 9 to 12 days ($n = 8$).



Figures 3, 4.—3. Marked male of the harvestman *Chavesincola inexpectabilis* taking care of eggs laid on a piece of tree fern trunk. The dotted circles indicate the position of the eggs. 4. Detail of the clutch after the addition of more eggs. Note that the eggs are covered by debris (photos by B.A. Buzatto). Scale bars = 5 mm.

Paternal care.—Non-guarding males and females were frequently seen walking around in the terrarium at night, and they were observed eating at least 10 times. Guarding males, on the other hand, rarely left their nests to forage at night; when they did ($n = 2$), they remained within 10 cm of the nest entrance. Additionally, unlike females that ate the cockroach pieces on the spot, guarding males and males that

were defending mud nests without eggs took the food to their nests before consumption ($n = 6$). In one case, a non-guarding male was observed entering a mud nest and seemingly trying to remove some eggs with his pedipalps, probably as an attempt of cannibalism. The guarding male (M1), which was 2 cm away from the nest entrance, attacked the intruder male using the first pair of legs and pedipalps. The non-guarding male left the nest

without cannibalizing any egg, and was chased by the guarding male for nearly 30 s. After that, the guarding male returned to his nest and remained with the fourth pair of legs blocking the nest entrance for nearly one hour.

DISCUSSION

When males are in charge of egg brooding, they become a reproductive resource for females and some degree of sex-role reversal may be expected (Owens & Thompson 1994; Parker & Simmons 1996). In such cases, male-male competition may be less intense and no sexual dimorphism is expected. Although most gonyleptids show strong sexual dimorphism, males being larger and more armed than females, this dimorphism in paternal species of the subfamilies Caelopyginae and Progonyleptoidellinae is very subtle. Females of many species have spiny legs and apophyses as long as those of males (e.g., Pinto-da-Rocha 2002), or in other cases, neither sex has any leg armature at all (e.g., Kury & Pinto-da-Rocha 1997). However, strong sexual dimorphism may be found among paternal species of the subfamily Gonyleptinae. In this subfamily, males of some species defend very specific sites (holes in roadside banks and trunks) as nesting sites, and leg armature seems to be involved in the defense of this scarce resource against other males (Machado et al. 2004). Males of *C. inexpectabilis* also defend nesting sites and, as could be expected, males have strong armature on the fourth pair of legs. They use the spines and apophyses of these legs to fight other males and to repel them from the nesting sites. Similarly to males of *Neosadocus* sp., which also occupy holes in roadside banks as nesting sites (see figs. 2B, C in Machado et al. 2004), males of *C. inexpectabilis* use the heavily armed fourth pair of legs to block the entrance of their nests and to pinch intruder males.

Most descriptions of courtship in harvestmen of the suborder Laniatores lack detailed information, such as which parts of the female body are touched by the male. Even though the courtship behavior of *C. inexpectabilis* follows the general pattern previously recorded for some gonyleptid harvestmen (see Machado & Macías-Ordóñez 2007), here we provide additional information showing, for instance, that males intensively touch the genital opening of the female. It is possible that these touches stimulate the female to open her genital opening, a prerequisite for male intromission among Laniatores. Unreceptive females clearly avoid male touches on the genital opening by lowering the venter to the substrate. On the other hand, receptive females allow the males to grasp them with their pedipalps and raise the front of their bodies so that penetration can occur. The end of the copulation is also apparently determined by the females, when they are able to release themselves from the intromission and from the pedipalpal grasping. In species of Eupnoi, the female may reject intromission, but grasping seems harder to avoid because the male tightly hooks his long, sexually dimorphic pedipalps to the base of female's legs II near the trochanter. Apparently, Eupnoi males rely more on the powerful grasping to initiate copulation with females, whereas Laniatores rely more on precopulatory courtship (discussion in Machado & Macías-Ordóñez 2007).

Post-copulatory courtship in *C. inexpectabilis* occurs as males tap on the dorsum and venter of females using their legs. Intense

female stimulation both during and after copulation may be viewed as a male strategy to increase the number of eggs fertilized and also increase paternity (Eberhard 1996). Additionally, the total time spent by ovipositing females inside a male's nest may reach four days, quite a long period when compared to other harvestman species (e.g., Juberthie & Muñoz-Cuevas 1971; Mora 1990; Machado & Oliveira 1998; Willemart 2001). In *Pseudopucroliia* sp., another Heteropachylinae species we are studying in our laboratory, males block the entrance of the nest with their bodies and also actively prevent females from leaving (Nazareth & Machado unpub. data). This coercive behavior, associated with repeated copulations, is possibly another male strategy to increase paternity and the number of eggs that one female will lay inside the nest.

The oviposition behavior of *C. inexpectabilis* is markedly different from that of other Laniatores, including representatives of the family Gonyleptidae (e.g., Juberthie & Muñoz-Cuevas 1971; Machado & Oliveira 1998; Willemart 2001). A unique behavioral feature is that females hold the eggs on the chelicerae before depositing them on the substrate. It is possible that females use secretions from the mouthparts to cover the eggs before their deposition on the substrate to promote the attachment of debris on them or to moisten them with anti-pathogenic compounds, as some centipedes do (Brunhuber 1970; Lewis 1981). The behavior of covering eggs with debris has been previously described for several harvestman species of the families Cosmetidae and Gonyleptidae that present no care or exclusive maternal care (references in Willemart 2001). The only cases of egg covering reported so far for a paternal species occur in the tryaenonychids *Karamea* spp. (Machado 2007), which are not closely related to the Gonyleptidae (Giribet & Kury 2007). This behavioral trait, therefore, clearly evolved independently in these two families, but in both cases might be related to egg protection by providing camouflage and/or preventing dehydration (Willemart 2001; Elpino-Campos et al. 2001).

Maternal egg-guarding is a costly behavioral strategy for iteroparous arthropods because it reduces lifetime fecundity by increasing the risk of death from predation and reducing foraging opportunities for guarding females during the long periods of care (Tallamy & Brown 1999; see also Buzzatto et al. 2007). Reduction of foraging is one of the main costs paid by guarding females and, according to the "enhanced fecundity hypothesis," exclusive post-zygotic paternal care may be viewed as a fitness-enhancing gift from males to females because it offers females two direct benefits: the cost-free care of their offspring and the freedom to forage for additional food (Tallamy 2001). After oviposition, eggs of *C. inexpectabilis* are left under the guard of the male, and females are released to forage and to produce more eggs. The intervals between two consecutive ovipositions ranged from 9 to 12 days, which is almost three times shorter than the interval between two ovipositions in the maternal gonyleptid *Discocyrtus oliverioi* Soares 1945, which was also studied in captivity where food was always available (Elpino-Campos et al. 2001; G. Machado, unpub. data). This interval is also 10–15 times shorter than the median interval between two ovipositions in three other maternal gonyleptids studied in the field (where food is supposed to be a limiting factor for female fecundity): *Bourguyia hamata* (Machado & Oliveira

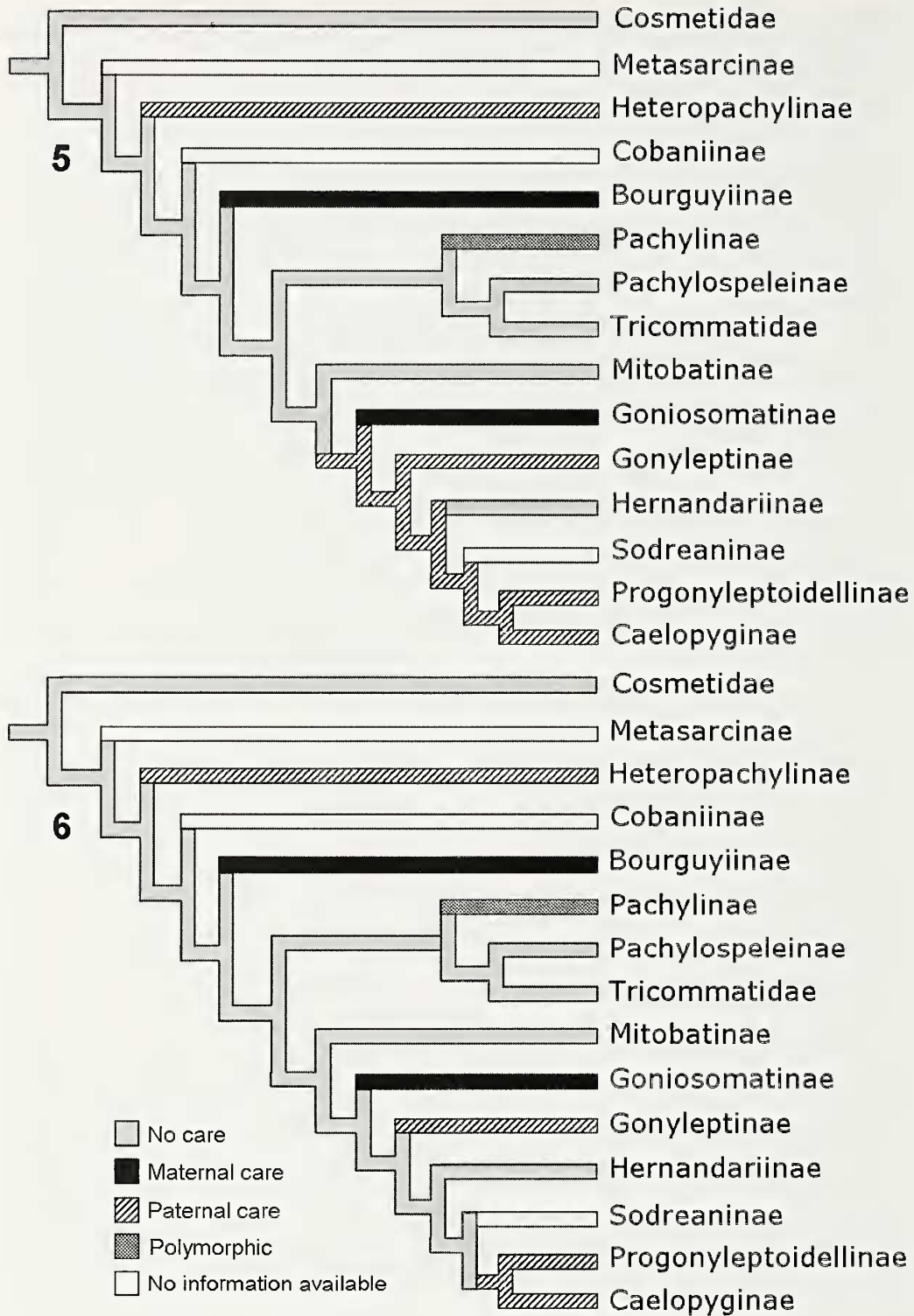


Figure 5, 6.—Internal phylogeny of the family Gonyleptidae (modified from Kury 1994 and Pinto-da-Rocha 2002) showing the forms of parental care presented by each subfamily. Behavioral data were mapped using the program Winelada (Nixon 1999), using ACCTRAN (5) and DELTRAN (6) optimization. Since there are no data on the internal phylogeny of some groups, the following assumptions were made: (1) most species of Cosmetidae do not care for the eggs (see table 12.2 in Machado & Macías-Ordóñez 2007) and the only case of maternal care reported so far in the family was considered as an autapomorphy (see Goodnight & Goodnight 1976 and Machado & Raimundo 2001); (2) although there is a great diversity in the forms of parental care within the subfamily Gonyleptinae (see table 12.2 in Machado & Macías-Ordóñez 2007), paternal care was tentatively considered as the plesiomorphic state in order to investigate how this polarity assumption could affect the optimization of this behavioral trait on the tree; (3) the information for the Pachylinae was considered as polymorphic because cases of no care and maternal care are evenly distributed in the species of this subfamily (see table 12.2 in Machado & Macías-Ordóñez 2007).

2002), *Goniosoma albiscryptum* (Willemart & Gnaspini 2004), and *Acutisoma proximum* (Buzatto et al. 2007). Apparently, the reproductive rate of *C. inexpectabilis* females is higher than females of species with maternal care, a likely consequence of their increased foraging rate, but experimental studies are necessary to address this question more carefully.

By mapping the available data about reproductive biology on the internal phylogeny of the Gonyleptidae, it is possible to infer that paternal care has evolved two or three times independently in the family, according to the type of optimization (Figs. 5, 6). Since the clutch and the nesting site of paternal species from the subfamilies Gonyleptinae and Progonyleptoidellinae + Caelopyginae are remarkably different (see discussion in Machado et al. 2004), we believe that DELTRAN optimization, which favors convergence, is the most appropriate scenario for the evolution of male care in the gonyleptids (Fig. 6). According to both Figs. 5 and 6, all cases of paternal care in gonyleptids are derived from no care. For the Heteropachylinae, however, this evolutionary transition should be interpreted cautiously because there is no published information on the reproductive biology of the Andean subfamily Metasarcinae and of the basal monotypic subfamily Cobaniinae. Data on the reproductive behavior of these two subfamilies are crucial to provide both a robust hypothesis about the plesiomorphic form of egg assistance in gonyleptids and a more complete scenario of the transitions between different forms of parental care in the family.

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